The Internal Sac of the Aedeagus of Podabrus (Coleoptera: Cantharidae)

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Intricate and fascinating are the shapes of the evaginated internal sac of the aedeagus in many species of some genera in the CANTHARIDAE. Similar interesting features of the internal sac have been found to occur in some genera in other families of beetles. These features indicate that potentially important differentiating characteristics are here to be found. Despite the fact that these characteristics have been so recognized in the past, too little effort has been made to utilize this organ in the description of or recognition of species. This is due largely to the problems of affecting the eversion of the internal sac.

A growing awareness by the senior author that the internal sac did offer valuable taxonomic characters made it requisite that further study of this organ be made.

History

The internal sac of the aedeagus has only recently been used as a factor in the separation of closely similar species in the Cantharidae. The senior author is at present attempting a reanalysis of the specific characteristics in the hitherto neglected organ. Prior to this time, the internal sac had been known to have possible taxonomic value but the use thereof had seldom been employed.

Sharp and Muir in 1912 noted and illustrated the complicated internal sacs of the aedeagae of *Silis ruficollis* and *Cantharis (Rhagonycha) limbatus*. Their study does not stress nor survey the importance of this organ in the family but merely notes it as being quite complex in the species cited.

McKey-Fender (1950) used the more or less deflated median lobe of the aedeagus and described briefly the internal sac ". . . membranous internal sac, which is armed apically with a flagellum composed of a bundle of long slender bristles," in her new subgenus *Cultellunguis* of the genus *Cantharis*.

Werner (1966) used the internal sac in studies of the Anthicidae. However his sketches indicate that they may have been made with the organ not everted.

Green (1966) commented briefly on the extreme importance of the everted internal sac of the aedeagus in the Cantharidae. He noted the difficulty in obtaining a fully everted condition, especially from dry cabinet specimens. Therefore, he confined his studies to the more strongly chitinized and "positively distinctive" aedeagal features. In this difficult genus (Silis), Green reveals some species complexes that he is unable to resolve. Whether or not the eversion of the internal

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sac would have solved these problems is not known. Studies of the future may determine this.

The senior author is at present involved in a revisional study of the genus *Podabrus*. In this study attempts are being made to utilize the everted internal sac of the male aedeagus towards a better understanding of this difficult genus. The junior author is occupied with the preparation of specimens for the work and experimentation with methods in search of the most satisfactory procedures for such preparation. If successful, attempts to utilize these methods with other poorly known genera of the Cantharidae will be made.

The Aedeagus of Podabrus

(Figs. 1, 2)

The male genital armature (aedeagus) is comprised of two major divisions: An enveloping protective tube, the *tegmen*, and the membranous inner tubular *median lobe*.

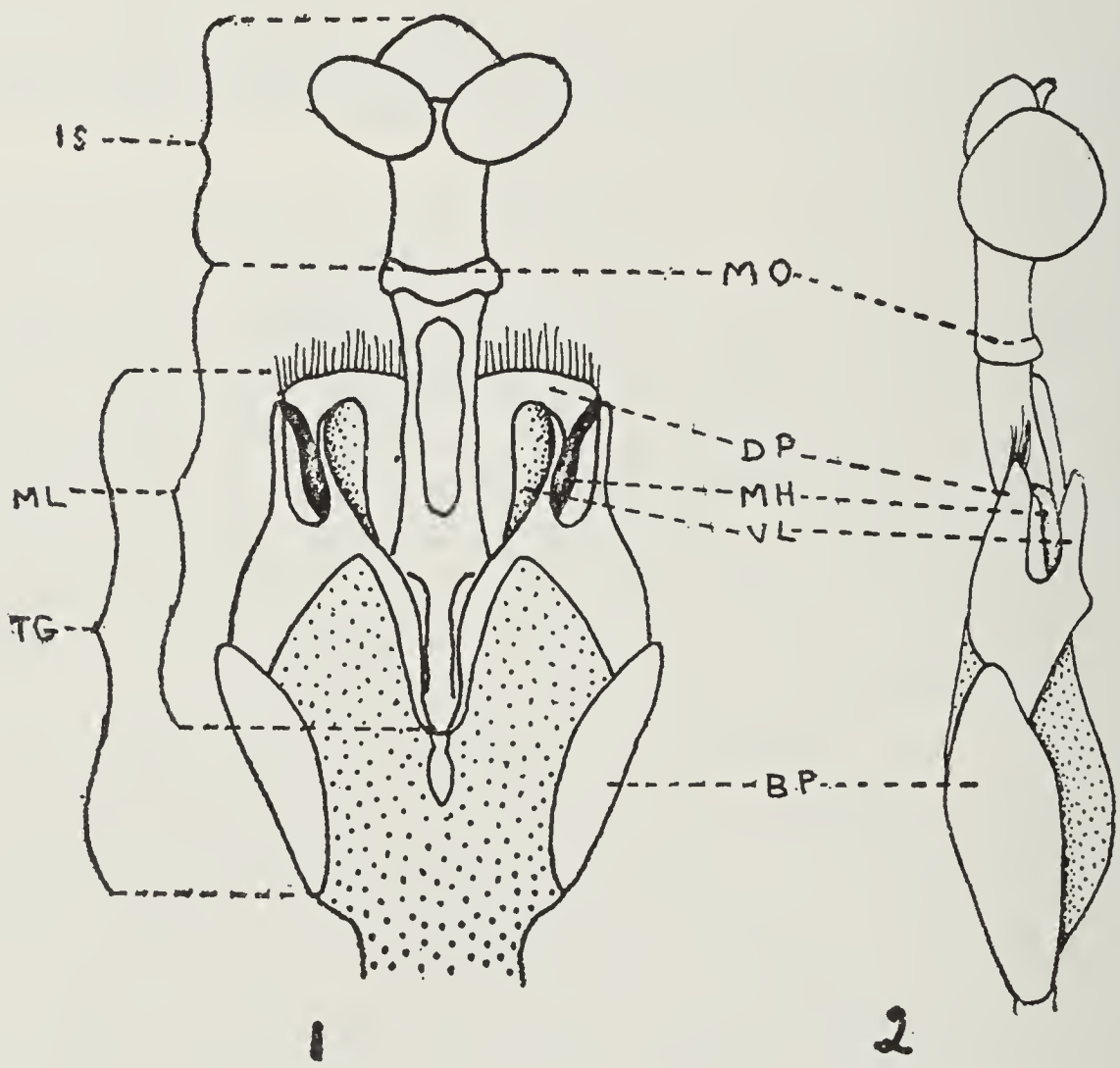
The tegmen possesses a pair of moderately strongly sclerotized *basal plates*, a *dorsal plate* that is membranous with the apical margin more or less widely sclerotized, and a pair of sclerotized *ventral lobes*.

The *basal plates* are circular to subovate, investing only the proximolateral portions of the tegmen. They offer no obvious distinguishing characteristics.

The *dorsal plate* may have the apical margin rounded, truncated or more or less widely deeply emarginate. It may or may not have an apical fringe of long hairs. If not having an apical fringe, the inner surface may or may not be clothed with moderately sparse erect hairs. These characters of the dorsal plate may be and often are diagnostic. The *ventral lobes* are the more or less strongly produced lobes of the latero-ventral sclerites. These sclerites rise from beneath the distal edge of the basal plates. They are produced inwardly on the ventral face until they meet for a short distance, usually with a short suture or trace thereof at the juncture. Ventrally these plates diverge apically, forming a deep, more or less V-shaped incision. Laterally these plates extend to connect with the ends of the sclerotized apex of the dorsal lobe. Laterally there is a deep, more or less U-shaped incision on each side. The ventral lobes thus formed may be in the shape of rather broad plates apically or more or less compressed and contorted lobes. They may or may not attain the distal margin of the dorsal plate or they may extend beyond it. The ventral lobes similarly offer good diagnostic characters in some species.

Inside the tegmen is the *median lobe*. In many species it contains, on its dorsal face, a heavily sclerotized laterally produced pair of parameres, the *median hooks*, united at their bases. The apical portions of the median hooks are bent up and more or less produced distally, becoming visible in the lateral apical incisure of the tegmen. In some species the median hooks are rudimentary, evidenced only by sclerotized patches on the median lobe. The median hooks frequently offer good characteristics.

Invaginated in the distal end of the median lobe, through a *median orifice,* is the *internal sac.* This, the intromittent portion of the aedeagus, becomes everted and fully extended in copulation. In the Cantharidae it best displays itself when in this state of eversion and extension. Otherwise it is of little value as an aid to the recognition of or differentiation of species. The extended internal sac is generally comprised of a shaft that may be long and slender, to short and broad, and a head that may be small to massive. Both the head and the shaft may be moderately simple but usually one or both are complexly ornamented with diverticula of varying sizes, shapes and numbers, variously located on the organ. On either the head or shaft or on their diverticula may be found setigerous areas in which the density and length or stoutness of the setae may vary from area to area. Inside the everted internal sac are a pair of longitudinal struts. These may be quite evident or scarcely, if at all, discernible. As viewed laterally, they may be median or on the ventral or dorsal surface. As viewed ventrally, they may be paired and separated, paired but connate medially or fused into a broad plate. It is the internal sac that should in future studies be found to possess the best diagnostic characteristics.



Figures 1 & 2. Aedeagus of *Podabrus punctatus* LeC. (internal sac evaginated). Fig. 1, ventral view. Fig. 2, lateral view. Legend: BP-basal plate, DP-dorsal plate, IS-internal sac, MH-median hooks, ML-median lobe, MO-median orifice, TG-tegmen, VL-ventral lobe.

Methods and Materials

As the present studies are pretty much pioneer in nature, many problems have been encountered that have perforce been faced with a rather hit or miss ap-

proach. The senior author began the work anticipating that some colleague had faced similar problems and could offer some clues or formulae that would simplify his tasks. Unfortunately no one was found who could offer such assistance; therefore a trial and error approach was initiated with frequent unsatisfactory results. A number of problems became immediately evident: 1. The most satisfactory method of removal of the aedeagae from the specimens, both freshly collected and dried cabinet; 2. The best procedure for the eversion of the internal sac in as nearly a normal condition as possible; and 3. The best method of preservation and storage of the prepared aedeagae with the everted internal sac intact.

Green (1966) recommends the relaxation of the males of *Silis* for three days in a very damp relaxing chamber. Following relaxation the removal of the aedeagus is accomplished by cutting the apical abdominal sternites medially throughout their length. The aedeagus is then removed, glued to a triangle and pinned beneath the specimen.

Sharp and Muir (1912) had success in dissection by placing the aedeagus in a weak caustic potash for a time and thus swelling the muscles. Through the insertion of a very fine syringe into the median foramen and the gentle application of localized pressure, they managed to evaginate the internal sac in a manner approximating the normal state.

Their process was tried by the senior author but without too much success. More attempts, using other more or less similar forms of preparation, were made but were equally unsuccessful. Despite these failures, continued experimentation is being done with this method.

The occasional good example with a well evaginated internal sac was hardened and placed on a slide in Canada Balsam as a means of preservation. This proved to be unsatisfactory. The organ immediately collapsed, possibly due to the weight of the cover glass or the consistency of the Balsam. The authors prefer the most rapid and satisfactory methods available for the preparation of their specimens. Toward this end, considerable experimentation was done and after over a year of disappointments, a surprisingly simple process was evolved that is by far the most satisfactory found to date.

In this method the beetle is relaxed in a small container. We have found aluminum ¼ to ⅓ cup measures satisfactory for this. The measure is nearly filled with cold water, the beetle placed in the water which is then brought to a vigorous boil and the specimen permitted to be agitated in the boiling water from two to twelve minutes, usually depending on the size of the beetle. By the time the water has started to boil, the specimen is so relaxed that there is no danger of breakage.

The beetle is next removed from the water and placed on a blotter to remove any excess water. It is then placed on its back, under the microscope and still on the blotter, with the tip of the abdomen towards the operator. The aedeagus is removed by pressing down with an insect pin or dissecting needle medially of the abdomen. The pressure is carefully advanced towards the abdominal apex with a rolling movement, forcing the aedeagus to near the apex or even pushing it en-

tirely out. Extreme care must be employed in this procedure that the pressure be applied to the base of the aedeagus and not the ventral surface, thus avoiding possible distortion of the aedeagus. When maximum extension has been attained by this means, an insect pin can be inserted under the apical edge of the 7th sternite near the lateral margin, thence carefully worked in behind the aedeagus and used as a lever to force it out.

Quite frequently the boiling and agitation of the beetle, also produces eversion of the internal sac of the aedeagus. In the event of complete eversion it is desirable to return this organ to the water before it has an opportunity to dry and shrink. It will there remain terete and extended until it can be preserved in a vial of alcohol. Preserved, the vial and beetle should each be given the same accession number, or some form of identification so that if they become separated, they can readily be reunited.

The authors are using an accession system as indicated above, the vials, upright in racks, are kept apart from the beetles. An accession number is applied to each vial. The beetles are kept in Schmidt type insect boxes, each beetle with its accession number on a label on the insect pin beneath the locality and collectors labels. This system is used to avoid the continuous setting and releasing of the aedeagae from points upon which they will be affixed following the completion of the study.

Once the internal sac has been everted, it can be allowed to dry. Tests have been made on specimens of several species to ascertain whether or not the everted internal sac can successfully stand repeated drying and boiling. In all instances, the drying and shriveling of a fully everted sac seems not to damage it at all or very little. One specimen was boiled and dried and boiled again 15 times and was just as well produced following the fifteenth boiling as it was following the first. Others have been carried through four or five such repeated operations without any seeming damage. This repetition is not recommended as it is quite possible that too vigorous reboiling might burst or damage an otherwise excellent specimen.

Comments and Explanations

Although the value of the aedeagus as an aid to the identification of species has long been recognized and used, stress has been towards the more readily accessible and more strongly chitinized tegmen of the organ. The membranous internal sac that usually must be everted and inflated to be studied is something else. Many consider that this procedure is too complicated and difficult for general use and that many collectors, especially amateurs would quit, rather than go to such extremes to attempt to determine their species.

This is quite true and it is not the intent of the authors to propose such use. Such advanced procedures as the use of the evaginated internal sac would be confined to the studies of the specialist or revisor. In reality, the senior author is hopefully using this means to help him understand this genus and its species; to help him assess the exterior characteristics that have in the past been utilized to separate the genus into groups; to help him determine what exterior characters (if

any) are valid for the differentiation and recognition of species; and to comprehend its relationship with its most closely allied genera.

To illustrate some of the problems that occur in the recognition or identification of closely allied species, the following examples are offered.

In some instances, the coloration may vary considerably, other conditions being similar (i.e., sculpture, shape, size, etc.). Even the tegmen of the aedeagus may be so similar that differences are not discernible. In these instances the internal sac can be a valuable aid to the determination of the possible existence of more than one species.

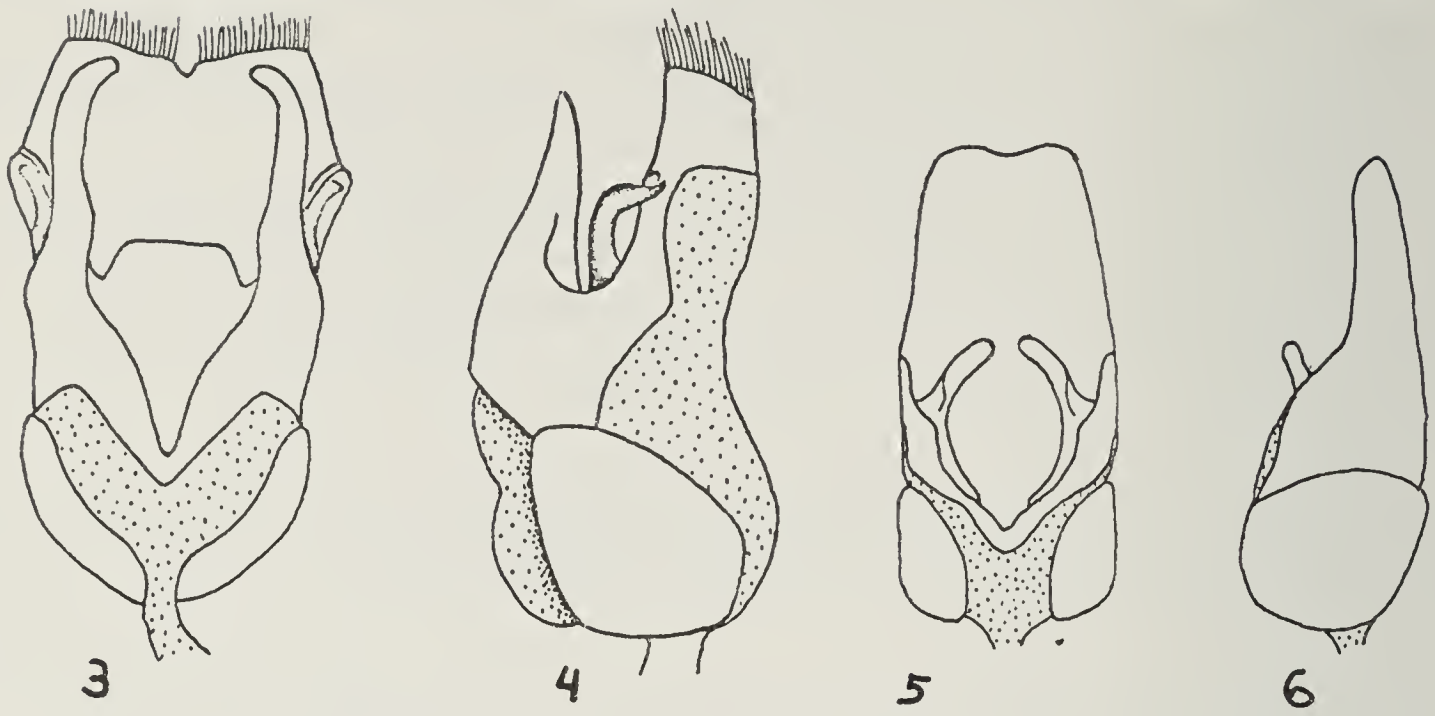
As demonstrated by Green (1947), *Podabrus knobeli* Lec. and *P. frosti* Fend. vary externally to the extent that they are inseparable but they can readily be distinguished by differences in the tegmena. Conversely *P. comes* Lec., *P. pruinosus* Lec. and *P. gradatus* Lec., all are readily separated on the basis of color, but have been united all as subspecies of *P. pruinosus,* this being the only character for separation. The tegmena of these show no discernible differences.

P. knobeli Lec. and *P. frater* Lec. are differentiated on the basis of color and the presence on *P. frater* or a more or less evident brush of hair on the mesotibiae (vestiture normal on *P. knobeli*). In these two species, the tegmena are indiscriminate. However the internal sacs validate these two species.

There are one or two species of *Podabrus* wherein attempts to obtain eversion have been unsuccessful despite adequate series for such eversion. The reason for this failure has at present not been determined. It may be that the degree of difficulty of eversion, or impossibility thereof, is a specific characteristic; that these species have reduced median orifices, the edges of the median orifices are less elastic or that the internal sacs are less pliant.

The system described above is not necessarily recommended for all families of the Coleoptera. It has proven decidedly workable with most species of the genera *Cantharis* and *Podabrus* of the CANTHARIDAE. Unsuccessful attempts have been made to obtain eversion of the internal sac by this process in the genera *Ellychnia* and *Pterotus* of the LAMPYRIDAE, *Chauliognathus* of the CANTHARIDAE, and *Cyphon* of the HELODIDAE. The median orifice in *Cantharis* and *Podabrus* is comparatively large and the margins might well be rather elastic thus permitting the more ready eversion of the internal sac. The simplicity of the system does suggest that attempts to apply it to other groups of beetles towards a better understanding of such groups are advisable. The senior author feels that there may well be more than one very similar species represented in some groups, species that to now have been merged under one name, and that a study of the internal sac will determine this whether pro or con.

Studies of the tegmen of the aedeagus have proved that keys to *Podabrus* of Fall and Fender, based as they are on male ungual characters (i.e., claws broadly cleft, narrowly cleft, toothed at base or some combination of these) whereas generally successful, are artificial and lead to unrealistic groupings of the species. These findings have been confirmed by the conformations of the internal sac. Fall's primary key point had seven divisions based on these ungual characters. Fender



FIGURES 3 and 4. Aedeagus of *Podabrus sp.* nr. *dreisbachi* Green with median hooks exposed. Fig. 3, ventral view, Fig. 4, lateral view. FIGURES 5 & 6, Aedeagus of *Podabrus lateralis* LeC. (?) with median hooks reduced and concealed. Fig. 5, ventral view. Fig. 6, lateral view.

recognized eight groups, similarly based. In Fender's group I, all claws of both sexes are broadly cleft; all species having the median hooks of the median lobe exposed (figs. 3, 4). In groups III to VIII, the males have at least the protarsal claws narrowly cleft and some of the remaining claws toothed; all species having the median hooks of the median lobe reduced and concealed (figs. 5, 6). In group II, the males have all claws with a short basal tooth. The larger species of group II have genitalia similar to those of group I, whereas the smaller species have genitalia like those of groups III to VIII. This indicates that at least Fender's group II should in some way be further divided to accommodate this separation. Whether or not this would be sufficient has not as yet been determined.

Even should the method presented here be proven to be unsuccessful in some groups, it is felt that students of such groups might find it advantageous to seek some other method (possibly a modification of this) for study of the internal sac and that in many instances they might be able to resolve such species complexes as may be found to occur in these groups. The writers feel that their continued experiments and investigation may reveal some process that will permit the simple eversion of the internal sac in these groups where they have heretofore failed.

Enthusiastic reactions received from colleagues point to the desirability of the continuation of the work. The suggestion has been made that the studies be expanded to include other families. This will have to be a project of the future. Hopefully, the use of the characteristics of the everted internal sac may lead to the resolution of the species complexes that now perplex so many students of the Coleoptera.

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FIELD NOTES

MEREDITH, SOUTH CAROLINA. In the October 1967 issue of the University of Kansas Science Bulletin (vol. XLVII, pp. 145-313), Dr. H. L. Willis published a paper on the bionomics and zoogeography of tiger beetles of saline habitats in the central United States. In the discussion of *Cicindela togata* Laferté which occurs on South Carolina coastal saline flats he stated, "The town of Meredith, South Carolina could not be located."

Meredith, a locality name I used from 1925 to 1929 for many hundreds of herpetological and entomological specimens, was a station on a railroad no longer in existence. The locality was in Lee County, 12 miles from Bishopville and 15 miles from Sumter, near Manville on present day roadmaps, at the junction highways US 15 and South Carolina 441.

How this locality could have been used for *C. togata* is a mystery and is certainly an error. The nearest saline flats are ninety miles away. Habitats found at Meredith may be known from the tiger beetles collected there: *Megacephala (Tetracha) virginica* L., *M. carolina* L., *Cicindela repanda* Dej., *sexguttata* Fab., *punctulata* Oliv., *abdominalis* Fab., *trifasciata ascendens* LeC., *gratiosa* Guerin, and *scutellaris rugifrons* Dej.—O. L. Cartwright, U. S. National Museum

ATAENIUS FRANKENBERGERI Balthasar, a species mistakenly placed in synonymy with *At. sulcatulus* (Chevrolat) by Dr. E. A. Chapin in his Revision of the West Indian Beetles of Scarabaeid Subfamily Aphodiinae (Proc. USNM, vol. 89, 1940, p. 41), has been collected at Brazos, Texas; Gulfport, Mississippi; Dade Co., New Smyrna, and Key Largo, Florida. The Key Largo specimens were collected by L. J. Bottimer and R. E. Woodruff in dung in the nest of *Neotoma floridanus smalli* Sherman. The specimens have been compared with Dr. Balthasar's type.

Ataenius waltherhorni Balthasar is represented by a specimen labeled Everglade, Florida, May 1912 in Wm. T. Davis collection, Purdue University. This species is not a synonym of *At. darlingtoni* Hinton (synonym of *Ataenius picinus* Harold!) as suggested by Dr. Chapin in the same paper. The type of *waltherhorni* has also been examined.

Ataenius frankenbergeri and *At. waltherhorni* were described by Dr. Vladimir Balthasar in Zweiter Beitrag zur Kenntnis der neotropischen Scarabaeiden, Arbeit. Morph. und Taxon. Ent. Berlin-Dahlem, Band 5, Nr. 1, pp. 55-51, 1938.—O. L. Cartwright, U. S. Nat. Museum.